In the Specification

Line and page numbers with these instructions are the Applicants best judgement as to location based on the filing of previous amendments to the Specification and the line and page numbers in the original PCT application. It is possible, however, that the actual numbers may be slightly different that what is cited here.

Please replace the paragraph beginning at page 3 line 2 that starts with the words "Conventional techniques choose sets of markers" with the following amended paragraph:

Conventional techniques choose sets of markers to scan a chromosomal region by choosing markers according to each marker's chromosomal location within the region. In a set of microsatellite markers described in 1994 for use in linkage studies, the markers were approximately evenly spaced, with average spacing between markers being 13 centiMorgans. The markers were distributed approximately evenly across the entire human genome (all human chromosomes) and were also selected because genotype data at the markers for individuals could be obtained by a semi-automated method.[1] A recent (1998) linkage study of the disease schizophrenia used a set of 310 microsatellite markers distributed approximately evenly across the entire human genome with average spacing of 11 centiMorgans between markers.[2] In a recent (1998) simulation of linkage studies to defend the practice of two-stage genome scanning, markers were spaced evenly every 10 cM(centimorgans) in an initial, sparser, first stage scan and evenly every 1 cM in a followup, denser, second stage scan.[3] Following up positive linkage study results from chromosomal regions in a sparse, first stage scan with a second, denser scan that focuses on studying the regions with positive first-stage results is a common technique. In these conventional studies, as is common, markers were chosen to be about evenly spaced across the chromosomal regions studied. In this manner, as is conventional, a one dimensional structure such as an entire genome, a chromosome or a region of a chromosome is "covered" by markers in order to scan the entire genome, chromosome or chromosomal region with a linkage study. (These conventional techniques[1, 2, 3] are not admitted to be prior art by their mention in this background.) (There is a possibly confusing, double meaning, of the term "marker map". It should be noted that a set of markers distributed along a chromosomal region, chromosome, or genome for linkage studies is also sometimes referred to as a "marker map" for use in chromosomal scanning by linkage studies. <u>A set of markers used in a linkage study is also called a panel of markers or marker panel.</u> In addition, chromosomal or genetic maps of markers are also referred to as "marker maps".)

Please replace the paragraph beginning at page 5 line 11 that starts with the words "Characteristics of a new type of linkage test" with the following amended paragraph:

Characteristics of a new type of linkage test, the TDT (transmission disequilibrium test), were described in 1993. The inventor, R.E.McGinnis, was one of the authors of this reference.[7] In 1996, Risch and Merikangas argued that conventional linkage analysis has limited power to detect genes of modest effect. And Risch and Merikangas attempted to illustrate the increased power of association based linkage tests such as the TDT over other types of conventional linkage tests.[8] However, Risch and Merikangas' analysis was criticized by Muller-Myhsok and Abel as being based on the optimal assumption that the analyzed allele was the disease allele itself. Muller-Myhsok and Abel concluded that researchers should be aware that the power of association studies such as the TDT can be greatly diminished in more common, less optimal situations.[9] In their response to Muller-Myshok and Abels' letter, Risch and *Merikangas essentially agreed with the logic* of Muller-Myshok and Abels' criticism. Risch and Merikangas stated that to a large extent, the expectation with respect to linkage disequilibrium across the genome is uncharted territory [10]. ~~territory and that it was difficult to predict the~~ ~~*power of using a less dense map at that time*~~[[.[10]]]~~**The inventor's work, however, is a predictor of the power and success of a less dense map.**~~ (None of the references in this paragraph [7, 8, 9, 10] is admitted to being prior art with respect to the present invention by their mention in this background.)

Please replace the paragraph beginning at page 6 line 14 that starts with the words "The inventor's calculations and observations about the increased" with the following amended paragraph:

The inventor's calculations and observations about the increased power of the TDT in more common, less optimal situations led him to the conclusion that the power of linkage studies using the TDT is greatly increased under some conditions. Under some conditions, the power of the TDT in a linkage study using bi-allelic markers is greatly increased when each of one or more of the bi-allelic markers used in the study fulfill two criteria: (1) the allele frequencies of each of the one or more of the bi-allelic markers are similar (but not necessarily the same, or even approximately the same) as the allele frequencies of an unknown (or unidentified) bi-allelic gene causing~~bi-allelic gene causing~~ a disease under study; and (2) each of the one or more bi-allelic markers is in some degree of linkage disequilibrium with the gene. Thus for a typical linkage study using bi-allelic markers and the TDT, ***to increase the likelihood of conditions occurring that increase the power of the TDT in the linkage study, the bi-allelic markers used in the study are chosen so that the least common allele frequencies of the markers vary systematically over a range or subrange of least common allele frequency.*** This major conclusion of the inventor's research is quoted directly from his unpublished manuscript that was included with previously filed U.S. Provisional Patent Applications:"This example is typical and highlights perhaps the most important finding of this paper; namely the importance of using bi-allelic markers with heterozygosity similar to that of a bi-allelic disease gene. Indeed, since a majority of susceptibility loci may be bi-allelic, the judicious use of bi-allelic markers of both high, medium and low heterozygosity may be crucial in order to detect and replicate linkages to loci conferring modest disease risk." (page 25) (In this context the phrase "bi-allelic markers with heterozygosity similar to that of a bi-allelic disease gene" is essentially equivalent to "bi-allelic markers with individual allele frequencies similar to those of a bi-allelic disease gene" and "bi-allelic markers of both high, medium and low heterozygosity " is essentially equivalent to the phrase "bi-allelic markers whose least common individual allele frequencies are high, medium and low".)

Please add the following new paragraph after the paragraph beginning at page 7 line 27 that starts with the words "In addition, the inventor's calculations and observations":

In addition, the two-dimensional linkage study techniques do not necessarily favor using markers in a scan that are about evenly spaced along a chromosome as in the conventional techniques. *This is because conventional techniques suffer from a kind of one dimensional view or lack of depth perception.*

Please add the following new paragraph after the paragraph on page 8 line 11 which starts with the words "Some patents that are in the same general areas as versions":

Conventional linkage study techniques, including conventional association-based studies and linkage analysis, are essentially one-dimensional and have a one-dimensional perspective, and generally use a one-dimensional concept of closeness in terms of chromosomal location to attempt to achieve linkage disequilibrium, especially increased linkage disequilibrium with a nearby gene (or trait-causing polymorphism). (These essentially one-dimensional conventional linkage study techniques, including conventional association-based studies and linkage analysis, also favor bi-allelic markers with least common allele frequencies near 0.5.) It is well known that increased disequilibrium between a marker and linked trait-causing polymorphism increases evidence for linkage provided by association-based linkage tests such as the TDT. However, what has not been recognized is that the specific allele frequencies of the marker can also have an enormous impact on the strength of evidence for linkage. The new association-based linkage study techniques of this application have a two-dimensional perspective, and use the two dimensions of chromosomal location and allele frequency. These two-dimensional techniques are based on the mathematical observation (or principle) that association-based linkage tests are increased in power as the frequencies of a bi-allelic trait-causing polymorphism allele and positively associated bi-allelic marker allele become similar in magnitude. The new "systematic covering" of one or more two-dimensional CL-F points is based on using this mathematical principle to increase the power of an association-based linkage test to detect linkage when linkage disequilibrium is present between one or more (covering) markers and a trait-causing polymorphism located at a point. These techniques are effective for any genetic characteristic, and for markers or trait-causing polymorphisms that are not bi-allelic. By using the two dimensions of chromosomal location and allele frequency together, the power and systematic nature of association-based linkage studies is increased. (No conventional linkage study technique in this paragraph is admitted to being prior art with respect to the present invention by its mention in this background.)

Please delete the heading "**Summary**" on page 8 line 18.

Please delete the paragraph on page 8 line 19 that starts with the words "**Versions of the invention use a new, two-dimensional**".

Please delete the paragraph on page 8 line 23 that starts with the words "**Conventional techniques suffer from a kind of one-dimensional lack**".

Please replace the paragraph beginning at page 9 line 34 that starts with the words "The inventor discovered that when a bi-allelic marker" with the following amended paragraph:

The inventor discovered that when a bi-allelic marker and a bi-allelic gene are located close together on a CL-F map, then the power of association based linkage tests to detect linkage disequilibrium between the marker and a trait-causing gene (when present) increases greatly. In general, the closer (the smaller the covering distance δ) in terms of either frequency distance, chromosomal location distance, or both, the greater the power. Systematically covering a CL-F region that is the location of an unknown trait-causing bi-allelic gene with bi-allelic covering markers, therefore greatly increases the power of association based linkage tests to detect linkage disequilibrium (when present) between one or more of the covering markers and the gene.

Please add the following four new paragraphs, and three new Tables after the paragraph beginning at page 47 line 17 that starts with the words "In using this silicon chip or glass slide technology", but before the heading "**Industrial Applicability**" on page 47 line 30: (The third new paragraph contains two mathematical expressions, one for H and one for F.) Please put the Tables 1, 2 and 3 sideways on separate pages following the fourth new paragraph. Please devote these pages just to the Tables as the Tables are very large. Please put only Tables 1 and 2 sideways on a first single page and Table 3 sideways on a second single page that follows the first page with Tables 1 and 2. The first new paragraph to be added is just below:

The following three paragraphs, mathematical expressions for H and F, and Tables 1, 2 and 3 are excerpted from the inventor's published paper (McGinnis, R.E.: *Hidden Linkage: Comparison of the affected sib pair (ASP) test and transmission disequilibrium test (TDT)*. Annals of Human Genetics, 1998, vol. 62, pp. 159-179). This paper is now available free online to the public from the publisher, Blackwell, at no cost.

TDT power is increased by disequilibrium between a bi-allelic marker and disease locus, and is also markedly increased when the disease allele and positively associated marker allele have similar population frequencies (p. 160). ***General algebraic model of linkage*** (p. 161) At the beginning of Results, I give expressions for P_s and P_t based on the following general model: A bi-allelic marker with alleles *A* and *B* is linked to a bi-allelic disease locus with disease-predisposing allele *D* and non-predisposing allele *d*. The model allows any penetrance for the *D/D, D/d* and *d/d* genotypes (α, β, and γ, respectively) such that $1 \geq \alpha \geq 0$, $1 \geq \beta \geq 0$, and $1 \geq \gamma \geq 0$, and also assumes that no other locus underlies disease susceptibility. The recombination fraction (θ) between marker and disease locus is variable as are the population frequencies of the four marker-disease locus haplotypes [f(AD) = c_1, f(Ad) = c_2, f(BD) = c_3, f(Bd) = c_4, where $c_1 + c_2 + c_3 + c_4 = 1$]. Note that once the haplotype frequencies are specified, the population frequency (p) of disease allele D is known ($p = c_1 + c_3$), as are the frequencies (m, 1 − m) of marker alleles A and B, respectively ($m = c_1 + c_2$; $1\text{-}m = c_3 + c_4$). Furthermore, the coefficient of disequilibrium (δ) equals $c_1 c_4 - c_2 c_3$ and thus, when convenient, the haplotype frequencies can be expressed as $c_1 = mp + \delta$, $c_2 = m(1-p) - \delta$, $c_3 = (1-m)p - \delta$, and $c_4 = (1-m)(1-p) + \delta$ (p. 161). Thus, $\alpha = r\gamma$ and the penetrance of D/d (β) can be considered to fall between α and γ by letting $\beta = \gamma + x(\alpha - \gamma) = \gamma + x(r-1)\gamma$ where x is a number between 0 and 1 (p.163). This framework generalizes the ASP-TDT comparison of Risch & Merikangas (1996) by encompassing many modes of inheritance rather than just one, and also by enabling TDT power to be calculated for a marker that is distinct from the disease locus (pp. 170, 171).

The expected proportion of A/B parents among those ascertained through an ASP can be shown to be H/F where H is as defined in Appendix I and

$$F = p^4\alpha^2 + 4p^3(1-p)\,{\frac{\alpha+\beta}{2}}^{2} + 2p^2(1-p)^2\beta^2 + 4p^2(1-p)^2\,{\frac{\alpha+2\beta+\gamma}{4}}^{2} + 4p(1-p)^3\,{\frac{\beta+\gamma}{2}}^{2} + (1-p)^4\gamma^2.$$

(Text and Expression for F above, from p. 169)

$$H = 2(c_1c_4 + c_2c_3)\; p^2\,{\frac{\alpha+\beta}{2}}^{2} + \tfrac{1}{2}p(1-p)\,{\frac{\alpha+2\beta+\gamma}{2}}^{2} + (1-p)^2\,{\frac{\beta+\gamma}{2}}^{2}$$
$$+ 2c_1c_3\{p^2\alpha^2 + \tfrac{1}{2}p(1-p)(\alpha+\beta)^2 + (1-p)^2\beta^2\}$$
$$+ 2c_2c_4\{p^2\beta^2 + \tfrac{1}{2}p(1-p)(\beta+\gamma)^2 + (1-p)^2\gamma^2\}.$$

(Expression for H above, from p. 174)

In concluding this section, I emphasize that Tables 1–3 show that when the disease locus and marker are bi-allelic, TDT power is substantially increased if the disease allele and positively associated marker allele have similar frequencies. Muller-Myshok & Abel (1997) independently made a similar observation, but they emphasized the weakness of TDT power when the m/p ratio departs from unity and δ is not close to δ_{max}. However, the tables illustrate that similar frequencies for the disease allele and associated marker allele can increase TDT power to reasonably high levels even when the m/p ratio substantially differs from 1 and δ is much lower than δ_{max}. For example, in Table 2 (r = 4), note that when $\delta = 1/2\ \delta_{max}$ and p = 0.15, a similar frequency (m = 0.25) for the disease-associated marker allele produces TDT power of 0.86 and P_t of 0.581; but when p = 0.15 and m = 0.5 at $\delta = 1/2\ \delta_{max}$, TDT power and P_t fall to 0.53 and 0.547, respectively. The difference in TDT power for these two situations can also be quantified by calculating the mean value of χ^2_{tdt} based on a sample of 200 ASP families and the values of P_t and H/F in Table 2 [i.e. $\chi^2_{tdt} = 800(H/F)\ (2P_t - 1)^2$]. When p = 0.15 and m = 0.5, $\chi^2_{tdt} = 3.53$ yielding a significance level of p = 0.06; but when p = 0.15 and m = 0.25, $\chi^2_{tdt} = 9.02$ for a significance level of $p < 0.003$. The large difference in significance level (0.06 versus 0.003) and power (0.53 versus 0.86) illustrated by this example indicates that careful attention to allele frequencies at bi-allelic markers may play an important role in future efforts to map susceptibility loci. (This paragraph is from p. 166 and 168; Tables 1 and 2 are from p. 165, and Table 3 is from p. 167.)

Table 1. *ASP and TDT power for $\alpha{:}\gamma$ penetrance ratio of $r = 2$*[a]

ASP test of fully informative marker[c]				TDT and ASP test of the same bi-allelic marker										
					$\delta = \delta_{max}$[b]					$\delta = \frac{1}{2}\delta_{max}$[b]				
			Power					Power					Power	
dis. allele freq(p)[b]	P_s	H/F	ASP	mkr. allele freq(m)[b]	P_t	P_s	H/F	TDT	ASP	P_t	P_s	H/F	TDT	ASP
$p = 0.60$	0.506	1.0	0.08	$m = 0.75$	0.568	0.511	0.33	0.60	0.08	0.532	0.507	0.35	0.19	0.07
				$m = 0.50$	0.560	0.510	0.50	0.67	0.09	0.530	0.507	0.50	0.22	0.07
				$m = 0.25$	0.537	0.506	0.40	0.26	0.07	0.519	0.506	0.39	0.10	0.07
$p = 0.40$	0.507	1.0	0.09	$m = 0.75$	0.553	0.509	0.34	0.42	0.07	0.525	0.508	0.36	0.13	0.07
				$m = 0.50$	0.573	0.513	0.50	0.83	0.10	0.536	0.509	0.50	0.30	0.08
				$m = 0.25$	0.563	0.511	0.43	0.65	0.09	0.534	0.508	0.40	0.23	0.07
$p = 0.15$	0.506	1.0	0.08	$m = 0.75$	0.526	0.505	0.36	0.14	0.06	0.513	0.505	0.37	0.07	0.07
				$m = 0.50$	0.537	0.507	0.50	0.32	0.07	0.518	0.506	0.50	0.11	0.07
				$m = 0.25$	0.565	0.512	0.42	0.67	0.09	0.534	0.509	0.40	0.23	0.08

[a] ASP power (1-tailed test) and TDT power (2-tailed test) for a significance level of 0.05 and sample size of 200 families; thus $n_{asp} = 400\ H/F$ and $n_{tdt} = 800\ H/F$.
[b] δ_{max} (δ_{min}) is most positive (most negative) value of disequilibrium for bi-allelic marker and disease locus with allele frequencies m and p, respectively; power results shown for δ_{max} ($1/2\delta_{max}$) at $m = 0.75$, 0.5 and 0.25 equal power results for δ_{min} ($1/2\delta_{min}$) when $m = 0.25$, 0.5 and 0.75, respectively.
[c] P_s for a fully informative marker is identical to P_s for a bi-allelic marker at $\delta = 0$.

Table 2. *ASP and TDT power for $\alpha{:}\gamma$ penetrance ratio of $r = 4$*[a]

ASP test of fully informative marker[c]				TDT and ASP test of the same bi-allelic marker										
					$\delta = \delta_{max}$[b]					$\delta = \frac{1}{2}\delta_{max}$[b]				
			Power					Power					Power	
dis. allele freq(p)[b]	P_s	H/F	ASP	mkr. allele freq(m)[b]	P_t	P_s	H/F	TDT	ASP	P_t	P_s	H/F	TDT	ASP
$p = 0.60$	0.516	1.0	0.16	$m = 0.75$	0.620	0.534	0.30	0.96	0.18	0.553	0.522	0.34	0.42	0.13
				$m = 0.50$	0.597	0.527	0.49	0.97	0.19	0.548	0.519	0.50	0.52	0.13
				$m = 0.25$	0.556	0.516	0.42	0.54	0.11	0.530	0.515	0.40	0.19	0.10
$p = 0.40$	0.525	1.0	0.26	$m = 0.75$	0.607	0.534	0.31	0.93	0.19	0.548	0.527	0.34	0.35	0.15
				$m = 0.50$	0.636	0.543	0.48	0.99	0.32	0.566	0.530	0.50	0.75	0.21
				$m = 0.25$	0.607	0.534	0.46	0.99	0.24	0.558	0.527	0.42	0.57	0.17
$p = 0.15$	0.530	1.0	0.33	$m = 0.75$	0.570	0.526	0.33	0.63	0.15	0.533	0.528	0.35	0.19	0.16
				$m = 0.50$	0.594	0.536	0.49	0.96	0.26	0.547	0.531	0.50	0.53	0.22
				$m = 0.25$	0.644	0.555	0.48	0.99	0.45	0.581	0.540	0.43	0.86	0.27

[a] ASP power (1-tailed test) and TDT power (2-tailed test) for a significance level of 0.05 and sample size of 200 families; thus $n_{asp} = 400\ H/F$ and $n_{tdt} = 800\ H/F$.
[b] δ_{max} (δ_{min}) is most positive (most negative) value of disequilibrium for bi-allelic marker and disease locus with allele frequencies m and p, respectively; power results shown for δ_{max} ($1/2\delta_{max}$) at $m = 0.75$, 0.5 and 0.25 equal power results for δ_{min} ($1/2\delta_{min}$) when $m = 0.25$, 0.5 and 0.75, respectively.
[c] P_s for a fully informative marker is identical to P_s for a bi-allelic marker at $\delta = 0$.

Table 3. *ASP and TDT power for $\alpha:\gamma$ penetrance ratio of $r = 10$*[a]

	ASP test of fully informative marker[c]			TDT and ASP test of the same bi-allelic marker										
					$\delta = \delta_{max}$[b]					$\delta = \frac{1}{2}\delta_{max}$[b]				
			Power					Power					Power	
dis. allele freq(p)[b]	P_s	H/F	ASP	mkr. allele freq(m)[b]	P_t	P_s	H/F	TDT	ASP	P_t	P_s	H/F	TDT	ASP
$p = 0.60$	0.527	1.0	0.28	$m = 0.75$	0.660	0.560	0.27	0.99	0.34	0.567	0.537	0.33	0.59	0.21
				$m = 0.50$	0.621	0.546	0.48	0.99	0.36	0.559	0.531	0.50	0.66	0.22
				$m = 0.25$	0.568	0.526	0.43	0.72	0.17	0.536	0.525	0.40	0.25	0.16
$p = 0.40$	0.547	1.0	0.59	$m = 0.75$	0.654	0.569	0.28	0.99	0.42	0.566	0.552	0.33	0.56	0.33
				$m = 0.50$	0.686	0.583	0.47	0.99	0.74	0.589	0.555	0.49	0.94	0.46
				$m = 0.25$	0.636	0.560	0.48	0.99	0.51	0.576	0.549	0.43	0.81	0.36
$p = 0.15$	0.580	1.0	0.94	$m = 0.75$	0.633	0.577	0.31	0.99	0.53	0.560	0.577	0.34	0.51	0.56
				$m = 0.50$	0.668	0.596	0.49	0.99	0.86	0.582	0.584	0.50	0.91	0.77
				$m = 0.25$	0.727	0.630	0.54	0.99	0.99	0.632	0.599	0.47	0.99	0.86

[a] ASP power (1-tailed test) and TDT power (2-tailed test) for a significance level of 0.05 and sample size of 200 families; thus $n_{asp} = 400\ H/F$ and $n_{tdt} = 800\ H/F$.
[b] δ_{max} (δ_{min}) is most positive (most negative) value of disequilibrium for bi-allelic marker and disease locus with allele frequencies m and p, respectively; power results shown for δ_{max} ($1/2\delta_{max}$) at $m = 0.75$, 0.5 and 0.25 equal power results for δ_{min} ($1/2\delta_{min}$) when $m = 0.25$, 0.5 and 0.75, respectively.
[c] P_s for a fully informative marker is identical to P_s for a bi-allelic marker at $\delta = 0$.

Please replace endnote X on page 48 with the following amended endnote:

X (1)Schuster, H. et al ~~(1995)~~(1996) Nature Genetics, 13(1) : 98 – 100.
(2)Gyapay, G. et al (1994) Nature Genetics, 7: 246-339.